REMARKS

Claims 1, 12, 14, 15, 28 and 47-50 are pending. Applicants have herein amended claims 1, 1 and 48, presented new claims 49 and 50, and canceled the previously pending claims 9 and 10. This amendment does not involve any issue of new matter. Entry of this amendment is respectfully requested.

Referring to the published specification, support for new claim 49 can be found, inter alia, at Paragraph 17 which provides "In certain preferred embodiments, the length of the dsRNA is at least 20, 21 or 22 nucleotides in length" and "In certain embodiments, the dsRNA construct is at least 25, 50... bases.".

Referring to the published specification, support for new claim 50 can be found throughout the application, including Figures 8, 18, 20, 22, and 26 and accompanying descriptions of those experiments describing the role of the RNase III enzyme Dicer in processing double stranded RNA substrates as part of the mechanism for inducing RNA interference.

I. Withdrawal of Application from Issue

In order to preserve the record in case of an appeal, Applicants hereby traverse the grounds and reasons given for withdrawing the current application from allowance. The current office action states that the withdrawal of the application from issue was to "correct the mistake of the [Patent] Office" which the office action states was a consequence to the PTO not considering a previously filed request for continued examination.

In April 2006, shortly after receiving the Notice of Withdrawal from Issue, the undersigned contacted Andrew Wang (who was the contact indicated on the Notice) to inquiry about the reasons underlying the decision to withdraw this application from issue. Examiner Wang first stated that the reason related to an organizational effort at the PTO to have all cases relating to RNA interference technology be handled by the same art unit, and because the current application was examined by another art unit, Examiner Wang stated that Art Unit 1635 wanted a chance to review this application. When the undersigned question whether such a ground was appropriate for withdrawing an application under 37 CFR 1.313(b), Examiner Wang then stated that the PTO had identified relevant prior art not previously considered in the instance application. However, the record does not indicate that this was indeed the case.

In the current office action, both references (i.e., Fire et al. U.S. 6,506,559 and Li et al. US 2002/0114784) relied on for purposes of rejecting the claims under 35 U.S.C. §102 were in fact *already of record* in this case, having been considered by Examiner Wilder in her decision to allow the application. The only new reference relied on in the current office action is the Graham et al. US Patent 6,573,099, which was cited under 35 U.S.C. §103. Applicants assert that this reference is no more than cumulative of the art already of record, and combined with the fact that the Graham et al. patent was not cited as being an anticipatory reference, would not have provided a reasonable or appropriate basis for withdrawing the application under 37 CFR 1.313(b). In fact, the search history of record (see PAIR entry dated "2006-09-06") suggests that the Graham et al. patent was not even identified by the patent office until a search conducted on August 14, 2006 – more than four months after the date of the Notice of Withdrawal from Issue. Accordingly, it does not appear that this reference served as a basis for withdrawing the application from issue.

Finally, Applicants note that the request for continued examination (RCE) filed in the instance application was at the request of the previous examiner, Examiner Wilder, with the explicit understanding from Examiner Wilder that the RCE was being filed in case she would not able to allow the application. That is, the RCE was merely intended to keep the application from going abandoned after a final office action if there were no allowance of the application and the examiner needed to raise additional grounds for why the application was not in condition for allowance. This practice is both encouraged by patent examiners and pervasive at the patent office to the point that no reasonable examiner could have construed the filing of an RCE as part of a response to a final office action as anything but what is alleged here. The fact that Applicants paid the issue fee in response to the Notice of Allowance makes this clear. The fact that the Applicants paid the issue fee also negates any ability of the patent office to allege that failure to consider the RCE was a mistake by the PTO that warranted correction.

Under the circumstances set forth herein and the file history, Applicants contend that the decision to withdraw this application under 37 CFR 1.313(b) was arbitrary and capricious, and respectfully request that the Notice of Withdrawal from Issue under 37 CFR 1.313(b) of 10 April 2006 be withdrawn, and that the Notice of Allowance and Fee(s) Due of 17 November 2005 be reinstated.

II. Rejection under 35 U.S.C. §112, second paragraph

The Office Action rejected claim 15 under 35 U.S.C. §112, second paragraph as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention. The Office Action states that it is unclear what one in the art would use as the basis for the five-fold reduction. In response, applicants respectfully traverse. Applicants submit that one skilled in the art would have understood from the context of the claim the basis for the five-fold reduction. Nevertheless, applicants without conceding the correctness of the Office Action's position but to expedite prosecution of the subject application have herein amended the claim to recite in part as follows: "compared to the expression of the endogenous gene in mammalian cells suspended in culture into which an expression vector encoding the hairpin RNA has not been introduced." Accordingly, the basis for the basis for comparison is even more clear. Applicants submit that this amendment obviates this ground of rejection and respectfully request reconsideration.

III. Rejection under 35 U.S.C. §102(e)

The Office Action rejected claims 1, 9-10, 12, 14-15, 28 and 47-48 under 35 U.S.C. §102(e) as allegedly anticipated by Fire et al. (U.S. 6,506,559) (the "'559 patent") and by Li et al. (US 2002/0114784) (the "'784 Publication"). These rejections are traversed.

(A) Neither the Fire et al. '559 patent nor the Li et al. '784 Publication qualify as Prior Art under 35 U.S.C. §102

(i) The generic teachings of the cited references do not anticipate the selected steps of the pending claims.

To anticipate, every element and limitation of the claimed invention must be found in a single prior art reference, arranged as in the claim. Karsten Mfg. Corp. v. Cleveland Golf Co., 242 F.3d 1376 (Fed. Cir. 2001); Scripps Clinic & Research Foundation v. Genentech, Inc., 927 F.2d 1565 (Fed. Cir. 1991). In this respect, both the courts and the US PTO have clearly articulated under which circumstances a prior art description of a genus can and cannot constitute an anticipation of a claim to a species under such genus. While the prior art need not describe the species *in haec verba*, the prior art disclosure must at least substantially identify the species and its properties to constitute an anticipation. If it is possible to derive a class of compounds of lesser

scope than the genus disclosed in a prior art reference on the basis of preferences ascertainable from the reference, anticipation may be found. Except under those circumstances, a genus does not constitute an anticipation of a species within that genus. The CAFC and its predessor court have plainly stated that "generic anticipation" is no in fact not anticipation at all. See, for example, In re Petering, 301 F.2d 676, 133 USPQ 275 (CCPA 1962); In re Ruschig, 434 F.2d 965, 145 U.S.P.Q. 274 (C.C.P.A. 1965); and M.P.E.P. §2131.02.

The standard articulated by the courts, and summarized in the M.P.E.P. at §2131.02, for whether or not the disclosure of a genus anticipates a claimed species was described more than 40 years ago in the CCPA decision of the In re Petering decision referenced above. In that case, the prior art disclosed a chemical genus having a limited number of substituent groups that represented either hydrogen or alkyl radicals, and an R group containing an OH group. The reference also disclosed a subgenus with preferred substituents for each group. The court held that the subgenus, containing only 20 compounds and a limited number of variations in the generic chemical formula, inherently anticipated a claimed species within the genus because "one skilled in [the] art would... envisage each member" of the genus. Thus, a genus anticipates a species if one of ordinary skill in the art is able to "at once envisage" the species compound within the chemical formula of the genus compound. In contrast to In re Petering, in the case of In re Ruschig (cited above), the court found that the rejection of a claimed compound in light of a prior art genus based on In re Petering was *not* appropriate where the prior art did not disclose a small recognizable class of compounds with common properties. Subsequent decisions have made it clear that this test for anticipation applies to other claim types, including method claims. See Akzo N.V. v. International Trade Comm'n, 808 F.2d 1471, 1 USPQ2d 1241 (Fed. Cir. 1986).

Neither the '559 Patent nor the '784 Publication provide the requested blaze marks that would otherwise permit one of ordinary skill in the art to be able to "at once envisage" the specific combinations of elements required in the presently claimed method. That guidance is found solely in the teachings of the present application. To the extent the cited art recite which cells are to be used, both references state that the corresponding methods can be used with cells of "any organism" - so in the range of 20-30 million different organisms including "plant, animal, protozoan, virus, bacterium, or fungus", either in vivo or in culture. Both references recite that double-stranded RNA can be 25-400 basepairs in length. The target genes can be endogenous or heterologous genes. On their faces, the cited references provide hundreds of millions, if not an infinite, variety of combinations and permutations. Neither reference provides any particular guidance that would lead one of ordinary skill in the art to "at once envisage" the claimed method – i.e., the use of an

expression vector to produce a hairpin RNA that, while attenuating expression of an endogenous genomic gene in cultured mammalian cells, does not produce sequence-independent killing of the mammalian cells.

(ii) The teachings of the cited references do not enable the method of the pending claims.

The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter. As is carefully set out in the M.P.E.P. at §2121.01, the "mere naming or description of the subject matter is insufficient". Prior art under §102(b) must, according to the CAFC, sufficiently describe a claimed invention *to have placed the public in possession of that invention.* In re Donohue, 766 F.2d 531 (Fed. Cir. 1985). The proper test of a publication as a § 102(b) bar is "whether one skilled in the art to which the invention pertains could take the description of the invention in the printed publication and combine it with his own knowledge of the particular art and from this combination be put in possession of the invention on which a patent is sought." In re LeGrice, 301 F.2d 929 (CCPA 1962). Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the invention with his own knowledge to make the claimed invention. In particular, one must be able to make the claimed invention without undue experimentation.

The determination of what level of experimentation is "undue," so as to render a disclosure non-enabling, is made from the viewpoint of persons experienced in the field of the invention. Neither the '559 Patent nor the '784 Publication would have placed the claimed method in possession of the public. Indeed, even Andrew Fire himself (the first named inventor of the '559 Patent) has made this admission in an article he published after the filing of the '559 Patent and about the time of the priority date of the present application. In the *review* article "RNA-triggered Gene Silencing" Trends in Genetics, 15: 358-363 (1999) attached as Exhibit A, which purports to be a survey of the state-of-the-art of RNA interference, Fire states at page 362-363:

> Procedures based on RNA-triggered silencing are now well-established tools for functional genomics of lower organisms (plants, invertebrates and fungi). Valuable information about gene function can be obtained, even in cases where only a partial loss-of-function is generated. From a technical perspective, one could certainly hope that RNA-triggered silencing would exist in vertebrates: this would facilitate functional genomics and might allow medical applications involving targeted gene silencing of 'renegade' genes. Although this hope is not ruled out by any current data, ***the simple protocols used for invertebrate and plant systems are unlikely to be effective.*** Mammals have a vehement response to dsRNA, the best-characterized

> component of which is a protein kinase (PKR) that responds to dsRNA by phosphorylating (and inactivating) translation factor EIF2a. *[emphasis added]*

At the time of the earliest priority date of the present application, there had been no reports that RNAi could be used in mammals. As pointed out in the Fire Trends in Genetics article, an accurate compilation of the state of the scientific art at that time, those skilled in the art of RNA interference believed that there were several potential limitations to its function in mammalian cells. Principal among these is that the accumulation of very small amounts of dsRNA in mammalian cells following viral infection results in the interferon response, which leads to an overall block to translation and the onset of apoptosis. Such considerations had discouraged investigators from using RNAi in mammals. That the statements by Andrew Fire are an admission of doubt in the ability of RNA-triggered silencing to work in mammalian cells is further evidenced by the statements made by Wianny et al., Feb 2000 Nature Cell Biology 2:70 attached as Exhibit B. The Wianny et al. paper, while published after the priority date of the present application, specifically cites the Fire Trends in Genetics article for raising the concerns "that RNAi might not work in mouse". See Wianny et al. at page 73, column two.

The statements made by Fire himself, several years after the filing date of the cited Fire et al. '559 Patent, demonstrate that the collective disclosures of the prior art up to the priority date of the present application, and including Fire's own disclosure in the '559 Patent, did not sufficiently describe the methods claimed in the present application so as to have placed the public in possession of that invention as required by §102(b). Fire admits that the protocols he himself has described and followed were not believed to be effective in mammalian cells. The plain fact is that neither the cited '559 Patent nor '784 Publication provide any guidance to overcome the perceived problems in mammalian cells described in the Fire Trends in Genetics review article.

There are an exceedingly large and impermissible number of combinations from the long laundry list of elements recited in the cited '559 Patent and '784 Publication that are inoperative. The vast majority of lengths covered for double-stranded RNA would kill cells from mammals and other vertebrates. Indeed, as set forth in the pending application and further articulated below, the combinations that one skilled in the art would be led to try by following the teachings of the '559 Patent or the '784 Publication would have killed mammalian cells as a consequence to the PKR response mentioned by the Fire Trends in Genetics article. The cited references also teach the use of RNA interference in many organisms in which the mechanism is not conserved, including many fungus and bacterium for which there is no evidence that RNA interference is possible.

To the extent that either the '559 Patent or '784 Publication can be read to provide any reasonable guidance as to which cells/organisms RNA interference was possible and what could be used as the inducing agent (i.e., the double stranded RNA), it is only when read with the benefit of hindsight using the teachings of the present application. As described in further detail below, the inventors of the present application are credited with elucidating the key points to the mechanism of action – including the role and mechanism of Dicer and RISC – that would be required to extend the teachings of the cited reference to cover the claimed method. Absent that understanding, those skilled in the art would have no reason to have selected any particular combinations that deviated materially from the working examples in the cited '559 Patent and '784 Publication.

The Patent Office has not met the burden of demonstrating that the disclosures of the '559 Patent and '784 Publication would give possession of the claimed method to a person of ordinary skill. In the instance of the cited '559 Patent and '784 Publication, the act of publication or the fiction of constructive reduction to practice will not suffice, as neither of those disclosures provide appropriate guidance to practice the method of the pending claims without engaging in undue experimentation.

IV. The claimed method is not obvious in view of the art

Applicants further submit that there was nothing in the prior art to teach or suggest attenuating expression of an endogenous genomic gene using the combination of the specific elements recited in the claims, namely using an expression vector to express a hairpin RNA that attenuated gene expression via an RNAse III dependent mechanism, and that the cell is a mammalian cell suspended in culture. The combination recited in the claims would not have reasonably been made, if made at all, prior to applicant's invention, because prior to applicants' filing, those skilled in the art, including an inventor of the cited '559 patent, did not think one could attenuate expression using an RNA interference mechanism in mammalian cells, let alone in mammalian cells suspended in culture, or understand the underlying mechanism for doing so. It was not until applicant's discovery of the underlying mechanism for RNA interference, as first described in applicants' specification, that those skilled in the art would have reasonably thought that RNAi could be applicable to mammalian cells, let alone mammalian cells suspended in culture. The argument for why one of skill in the art would have had no reasonable basis to arrive at applicants' claimed invention, prior to applicants' filing, is provided below.

Applicants submit that prior to their invention, one of skill in the art would not have reasonably believed that a double stranded RNA could induce sequence-specific gene silencing in mammalian cells. In order for those skilled in the art to have reasonably believed that a double stranded RNA could induce sequence-specific gene silencing, they first needed to understand the cellular mechanism of this biological phenomenon. At the time the '559 patent was filed in 1998, that mechanism was not known to the public nor described in the '559 patent, although procedures based on double stranded RNA-triggered silencing were fairly well-established tools for functional genomics of lower organisms (plants, invertebrates and fungi). Nevertheless, the simple protocols used for invertebrate and plant systems were not effective in mammalian cells at that time, as set forth above with reference to the cited article by Andrew Fire. Accordingly, even as late as September 1999 (*i.e.* later that the filing date of the '559 patent), Andrew Fire, one of the named inventors of the '559 patent, essentially conceded that understanding the underlying mechanism of using dsRNA to induce sequence specific gene silencing in mammalian cells was important, and his statements support the notion that he in fact did not understand such mechanism at the time.

In considering the effect of the cited references, it is important to also understand that it was necessary to know the mechanism by which RNA interference works in order to appreciate that hairpin RNAs could be used to induce gene silencing in mammalian cells. That is, in order for those skilled in the art to reasonably believe that a hairpin RNA could induce gene silencing, they first needed to understand the cellular mechanism by which double stranded RNA could induce sequence-specific gene silencing. As detailed in the Hannon and Hammond Declaration attached as Exhibit C, at the time of the filing of the cited of each of the '559 Patent and '784 Publication, that mechanism was not known to the public nor described in the cited references or other prior art teachings. The '559 Patent and '784 Publication are each entirely silent on the mechanism of RNAi.

Applicants submit that at the time the '559 Patent and '784 Publication were each filed, one skilled in the art would not have reasonably believed that one could inhibit expression of an endogenous genomic gene with an RNAi mechanism in cells suspended in culture. In particular, at the time the '559 Patent and '784 Publication were each filed, the ability of a few molecules of double stranded RNA to eliminate a much larger pool of endogenous mRNA had suggested a catalytic or amplification component to the interference mechanism. For instance, some of the plant literature favored an RNA-based copying system that was proposed to produce copious amounts of antisense RNA (while perhaps also producing additional sense and dsRNA). See Jorgensen et al. (1998) *Science* 279: 1486; Waterhouse et al. (1998*) Proc. Natl. Acad. Sci.* 95:13959 and

Wassenegger et al. (1998) *Plant Mol. Biol.* 37:349. This experience, along with similar observations in *C. elegans* (see, e.g., Fire et al. (1998) Nature 391:806), suggested an amplification process in whole organisms. That is, that RNA interference was the consequence of a systemic response. *If that had indeed been the mechanism, it would not be apparent or expected that RNA interference using double stranded RNA would work on cultured cells.* Accordingly, Applicants submit that one skilled in the art would not have expected to achieve attenuation of gene expression in cells suspended in culture using an RNAi mechanism.

At the time the cited patent references were each filed, one could certainly have hoped that RNA-triggered silencing would exist in vertebrates. However, the simple protocols used for invertebrate and plant systems were known not to be effective in mammalian cells. At that time, it was recognized in the art that there were several impediments to the use of RNAi in normal mammalian cells. Most mammalian cells harbor a potent antiviral response that is triggered by the presence of dsRNA viral replication intermediates. Reviewed Williams (1997). Biochem. Soc. Trans. 25, 509-513 and Gil (2000). Apoptosis 5, 107-114. In somatic cells, dsRNA activates a variety of responses. Predominant among these is PKR, a kinase that is activated by dimerization in the presence of dsRNA (Clarke et al. (1995) RNA 1, 7-20). PKR, in turn, phosphorylates EIF2α, causing a nonspecific translational shutdown. dsRNA also activates 2′-5′ oligoadenylate polymerase, the product of which is an essential cofactor for a nonspecific ribonuclease, RNase L. Reviewed in Baglioni et al. (1983). Interferon 5, 23-42. The ultimate outcome of this set of responses is cell death via apoptosis.

Applicants assert that in the absence of the biochemical and genetic approaches carried out by the inventors in several experimental systems and described in applicants' specification, those skilled in the art would have had no reasonable expectation that, based on the collective teachings of the prior art, hairpin RNA would have any effect as a gene silencing agent in mammalian cells. Moreover, prior to applicants' invention, one skilled in the art would not have believed that the claimed invention would work. *Applicants respectfully submit that the only way that one could read the '559 patent as teaching a dsRNA to attenuate expression in a mammalian cell in culture would be if one did not understand the literature; that is, if one were not one skilled the art.* Applicants further submit that the understanding of the mechanisms underlying RNAi in vertebrates came from the work of the present inventors, who identified the existence of conserved machinery for double stranded RNA-induced gene silencing from drosophila to mammals. The present inventors also defined the RNAi process as proceeding via a two-step mechanism. In the first step, double stranded RNA is recognized by an RNase III family nuclease called Dicer, which cleaves the

dsRNA into about 21-23-nt siRNAs (now called "small interfering RNA" or "siRNA" in the scientific literature). These siRNAs are incorporated into a multicomponent nuclease complex (RISC), which identifies substrates through their homology to siRNAs and targets these cognate mRNAs for destruction. Accordingly, based on the comments provided above, applicants submit that one skilled in the art would have had no reasonable basis to think that attenuation of gene expression RNAi would work in mammalian cells, based on the disclosure of the '559 patent, before applicants' filing.

Relative to double-standard RNA formed from two separate RNA strands, the notion that the prior art might suggest the use of hairpin RNA constraints would have encountered additional skepticism by those skilled in the art. As mentioned above, the state-of-the-art in RNA interference at the time the present inventions was made, while based on phenomology in insect and worms, suggested an amplification process that one skilled in the art could have conjectured to have occurred through a templated synthesis mechanism. Typical models for templated synthesis would have disfavored a hairpin relative to a duplex formed from two strands of RNA, as the duplex portion of a hairpin structure would be considered to be a more stable structure than a duplex formed from two separate strands, being harder to "melt" to form single stranded regions.

It is widely recognized that the inventors named on the present application elucidated the critical portion of the RNA interference pathway required to make the claimed method. As indicated in Exhibit D, Greg Hannon was recently awarded the "NAS Award in Molecular Biology" by the National Academy of Sciences "for elucidation of the enzymatic engine for RNA interference." This award recognizes the extraordinary scientific achievement and fundamental contribution that Greg Hannon made to the field of RNA interference – a fundamental contribution that was required for and gave to rise to the claimed method of the current application. Exhibit E is a copy of a slide presented by Craig C. Mello during his Nobel Lecture December 8, 2006, at Karolinska Institutet in Stockholm. This slide shows the Nobel Laureate Craig Mello acknowledging Greg Hannon's role in elucidating the fundamental part of the RNA interference pathway that gave rise to the claimed method of the present application as well as which moved the prior art understanding of this as a phenomenology to one in which other RNA species could be predictable used to cause silencing and in which organisms.

With respect to the use of expression vectors for producing hairpin RNA, as recited in the pending claims, there would have been yet additional reasons for those skilled in the art to have doubted that the teachings of the prior art could be modified to effect gene silencing. In particular, prior to the present application, one skilled in the art would have been aware that while double-

stranded RNA is often formed in the nuclei of mammalian cells, in this compartment it does not induce the effects characteristic of cytoplasmic double-stranded RNA. Rather, various publications at that time had suggested that nuclear double-stranded RNA is a target for the ADAR class (adenosine deaminases that act on RNA) of enzymes, which deaminate adenosines to inosines, resulting in partial or full unwinding. See, for example, Kumar et al. 1998 Microbiology and Molecular Biology Reviews 62(4):1415-1434; Kumar and Carmichael, 1997 Biochemistry 94:3542-3547; Bass et al. 1997 Trends Biochem Sci 22:157–162. ADARs were also known to be ubiquitous in the animal kingdom (Wagner et al. 1990 Mol. Cell. Biol. 10:5586-5590).

ADARs, which act in the nucleus, require duplex secondary structure in their target RNA substrates. Viral RNA genomes provided some of the first reported instance of A-to-I RNA editing was observed (Cattaneo et al. 1988 Cell 55:255–265). In certain instances, when the substrates for ADARs are extensive duplexes, up to 50% of adenosines are converted to inosine. Such promiscuous editing has been proposed to have roles in gene regulation, viral defense (Bass & Weintraub, 1988 Cell 55:1089–1098) and in biased hypermutation and persistent infection of certain viruses (Bass, 1997 Trends Biochem Sci 22:157–162). The consequence to this editing can include loss of basepair matches as well as retention of the RNA in the nucleus.

In sum, Applicants submit that based on the literature as of applicants' filing date, there was nothing to suggest attenuating expression of an endogenous genomic gene using the combination of the specific elements recited in the claims, including that the cell is (i) a mammalian cell, and (ii) is suspended in culture. Accordingly, applicants' claimed invention is novel and non-obvious over the disclosure of the '559 Patent and '784 Publication.

V. Antedating of Li et al. Published Application

The '784 Publication was filed 4 January 2002, and claims priority to, as a continuation, to USSN 09/493,301 filed 28 January 2000, which claims priority to two provisional applications, US Provisional Application No. 60/175,440 filed 11 January 2000 and US Provisional Application No. 60/117,635 filed 28 January 1999. Neither of the provisional applications contain the reference to the hairpin constructs referred to in the '784 Publication. Copies of these provisional applications can be submitted at the Examiner's request. Accordingly, the effective date of the '784 Publication, for purposes relied on by the Examiner, is 28 January 2000.

As evidenced by the attached Declaration of Gregory J. Hannon and Scott Hammond under 37 CFR §1.131 (the "131 Declaration" attached as Exhibit C) and the exhibits attached thereto, the

inventors had possession of the subject matter allegedly disclosed in Li et al. before the January 28, 2000 effective filing date of the '784 Publication. The 131 Declaration antedates the '784 Publication , thus removing the '784 Publication as citable art and thereby obviating the rejection under 35 U.S.C. §102(e). Reconsideration and withdrawal of this rejection are respectfully requested.

VI. Rejection under 35 U.S.C. §103(a)

The Office Action rejected claims 1, 9-10, 12, 14-15, 28 and 47-48 under 35 U.S.C. 103(a) as allegedly unpatentable over Graham, US Patent 6,573,099 (the "'099 patent"). The Office Action concedes that the '099 patent does not disclose inhibition in cells suspended in culture, or mammalian, primate or human cells, specifically. The Office Action states that the invention described in the '099 patent is for inhibition of targeted genes in an organism to defend against infection of viruses and also to treat disease, and thus, it would have been obvious to one in the art that mammalian, primate and human cells are clearly comprised in the scope of the teachings of Graham since the viruses targeted are bovine and human. The Office Action further states that it would have been obvious for one in the art to perform the inhibition methods in cells in culture before the use of the methods in cells in an organism such as a human since on in the art would have recognized the savings in cost and further since on the art would clearly be required to test such methods in an appropriate cell and animal model before testing in a human.

In response, applicants respectfully traverse. Based on the Office Action, it appears that the Office's position is that it would have been obvious *to try* applicants' claimed invention. In particular, the Office Action argues that "[i]t would have been obvious to one in the art that mammalian, primate and human cells are clearly comprised in the scope of the teachings of Graham since the viruses targeted are bovine (BEV) and human (HIV-1). However, applicants respectfully note that one of the requirements of a prima facie case of obviousness is that there must be a reasonable expectation of success. *See* MPEP 2143. The '099 patent was filed June 19, 1998, claiming priority to March 20, 1998. Applicants submit that, as argued above for the '559 Patent and '784 Publication, there would not have been a reasonable expectation of success in practicing applicants' claimed invention as of 1998 based on the disclosure of the '099 patent and what was known to one skilled in the art.

To reiterate what has been argued above, as of 1998, there would have been no reasonable expectation of success for inhibiting expression of an endogenous genomic gene using RNAi in

cells suspended in culture. In particular, at the time the '099 patent was filed, the ability of a few molecules of double stranded RNA to eliminate a much larger pool of endogenous mRNA had suggested a catalytic or amplification component to the interference mechanism. For instance, some of the plant literature favored an RNA-based copying system that was proposed to produce copious amounts of antisense RNA (while perhaps also producing additional sense and dsRNA). See Jorgensen et al. (1998) *Science* 279: 1486; Waterhouse et al. (1998*) Proc. Natl. Acad. Sci.* 95:13959 and Wassenegger et al. (1998) *Plant Mol. Biol.* 37:349. This experience, along with similar observations in *C. elegans* (see, e.g., Fire et al. (1998) Nature 391:806), suggested an amplification process in whole organisms. That is, that RNA interference was the consequence of a systemic response. If that had indeed been the mechanism, *there would not have been any reasonable expectation of success that RNAi using ds RNA would work on cultured cells*.

Second, there would have been no reasonable expectation of success for inhibiting expression of an endogenous genomic gene using RNAi in mammalian cells. In order for those skilled in the art to reasonably believe that a double stranded RNA could induce sequence-specific gene silencing, they first needed to understand the cellular mechanism of this biological phenomenon. At the time the '099 patent was filed in 1998, that mechanism was not known to the public nor described in the '099 patent, although procedures based on double stranded RNA-triggered silencing were fairly well-established tools for functional genomics of lower organisms (plants, invertebrates and fungi). Nevertheless, the simple protocols used for invertebrate and plant systems were not effective in mammalian cells at that time, as admitted by Andrew Fire as set out above.

The above-cited Wianny et al. reference, published after the filing date and publication date of the '099 patent, provides further evidence that there was no reasonable expectation of success for gene attenuation in mammalian cells as of the filing date of the '099 patent. In particular, Wianny states that "[s]o far there has been no report that RNAi can be used in mammals. Moreover, there are several indications of potential limitations to its function in this group of animals. Principal among these is that the accumulation of very small amounts of dsRNA in mammalian cells following viral infection results in the interferon response, which leads to an overall block to translation and the onset of apoptosis. Such considerations have discouraged investigators from using RNAi in mammals" (page 71, left column, lines 4-12).

Accordingly, Wianny provides further evidence that the literature suggested that one skilled in the art would have had no reasonable expectation of success in practicing applicants' claimed

invention as of February 2000 (*i.e.*, Wianny's publication date), let alone as of the filing date of the '099 patent.

Thus, in the absence of the biochemical and genetic approaches carried out by the inventors in several experimental systems and described in applicants' specification, those skilled in the art would have had no reasonable expectation that, based on the teachings of the '099 patent, single self-complementary RNA strands would have any effect as a gene silencing agent in mammalian cells.

Applicants further submit that the understanding of the mechanisms underlying RNAi in vertebrates came from the work of the present inventors, who identified the existence of conserved machinery for double stranded RNA-induced gene silencing from drosophila to mammals. They also defined the RNAi process as proceeding via a two-step mechanism. In the first step, double stranded RNA is recognized by an RNase III family nuclease called Dicer, which cleaves the dsRNA into about 21-23-nt siRNAs (now called "small interfering RNA" or "siRNA" in the scientific literature). These siRNAs are incorporated into a multicomponent nuclease complex (RISC), which identifies substrates through their homology to siRNAs and targets these cognate mRNAs for destruction.

Accordingly, based on the comments provided above, applicants submit that the '099 patent does not render obvious applicants' claimed invention. Applicants respectfully request reconsideration.

VII. Secondary Considerations

The use of hairpin RNA species to affect gene silencing in mammalian cells, according to the methods of the pending claims, is based on several factors that were first recognized by the Applicants, and to whom that credit has been given by the National Academy of Sciences and Nobel Laureate Craig Mello. Among those factors are that mammalian cells contain the nucleolytic activity necessary to process hairpin RNA constructs and produce functional siRNA products. Furthermore, the use of short hairpin RNA constructs does not produce a general sequence-independent killing of the mammalian cells, e.g., does not trigger an acute-phase response and the activation of a PKR.

Moreover, as the examples of the present application demonstrate, there are certain unexpected benefits to using hairpin RNAs to cause RNA interference in mammalian cells. For

instance, the hairpin RNA constructs generally exhibit better reassociation kinetics in cells than equivalent duplex RNA. Perhaps even more significant, the Applicants have demonstrated that transgenic cell lines can be engineered to synthesize hairpin RNAs that exhibit a long-lasting suppression of gene expression that persists throughout cell divisions.

As set forth above, as of the priority date of the present application, it was generally known in the art that the embodiments suggested from work in invertebrates, such as is the case of the various cited references relied on by the Examiner, did not work in mammalian cells. Rather than induce sequence-dependent suppression, the long dsRNA constructs most favored in invertebrates induced sequence-independent cell death. In considering whether a reference qualifies as prior, it is respectfully noted that when a prior art reference merely discloses the structure of a compound, evidence showing that attempts to prepare that compound were unsuccessful before the date of invention will be adequate to show inoperability. In re Wiggins, 488 F.2d 538, 179 USPQ 421 (CCPA 1971).

The above allegations notwithstanding, Applicants note that the prior art fails to teach or suggest the unexpected advantages arising from the combination of short hairpin RNAs for use in mammalian cells. Applicants contend that the combination of hairpin RNAs for use in mammalian cells has several unexpected advantages not taught or suggested by the teachings of Fire et al. To reiterate, such hairpin constructs: can be processed by mammalian cells to produce functional siRNA products; do not produce a general sequence-independent killing of the mammalian cells; exhibit favorable reassociation kinetics in cells; and can be used to engineer transgenic cell lines that exhibit a long-lasting suppression of gene expression that persists throughout cell divisions.

VIII. Conclusion

In view of the above amendment, applicant believes the pending application is in condition for allowance.

Applicant believes no additional fee is due with this response. However, if an additional fee is due, please charge our Deposit Account No. 18-1945, under Order No. CSHL-P02-010 from which the undersigned is authorized to draw.

Dated: 6 March 2007

Respectfully submitted,

By _____
Matthew P. Vincent
Registration No.: 36,709
ROPES & GRAY LLP
One International Place
Boston, MA 02110
(617)-951-7000
(617)-951-7050 (Fax)
Attorneys/Agents For Applicant